BIOLOGICAL BULLETIN

THE RÔLE OF THE FIN RAYS IN THE REGENERA-
TION IN THE TAIL-FINS OF FISHES.[1]

(IN *FUNDULUS* AND GOLDFISH.)

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The experiments which form the basis of this paper were planned for two purposes:

1. To reëxamine the facts, as set down by Morgan:

(*a*) The rate of growth is greatest where most material is needed to complete the typical form of the tail (1902) (*i.e.,* the controlling factors are not those usually considered as physiological ones—the availability of food or blood at the level of the cut —but certain formative factors).

(*b*) These formative factors are internal and may be expressed in terms of tension or pressure relations that initiate growth, govern the return to form, and cause a cessation of growth (1906).

2. To present, if possible, new data that would throw additional light upon the problem of regeneration and morphogenesis in the tail-fins of fishes.

Under the direction of Professor J. Walter Wilson, the experiments discussed in this paper were begun at the Arnold Biological Laboratory of Brown University (1927), and continued at the Marine Biological Laboratory at Woods Hole, Massachusetts (1928).

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HISTORICAL.

Broussonet (1786) recorded the following results of his ex-
periments in regeneration: (1) Fins reproduce themselves in slow
degrees; more rapidly in young fishes than in old; more rapidly
in some species than in others. (2) " Poissons dore's de la
Chine " regenerate fins: new rays can be distinguished after three
months; a severed " right fin " reaches original growth in eight
months; both ventral and caudal fins regenerate after oblique and
transverse cuts. (3) There is correlation between liability to in-
jury and power to regenerate (Bonnet's conception). (4) A part
of the " osselets " is necessary for regeneration; otherwise new
fins are not produced.

Unaware of Broussonet's work, Mazza (1890) found that re-
generation takes place in the tail of the goldfish (*Carassius
auratus*).

Weissman (1892) was aware of the fact that the Salamander
woud regenerate a lost limb, but did not believe that fins of fishes
would regenerate.

Unaware of the works of Broussonet and Mazza, Nussbaum
and Sidoriak (1900) published results of experiments in regenera-
tion on a young brook trout (*Salmo fario*), discussing, for the
first time, the histology of regeneration in tail fins.

T. H. Morgan (1900) was probably the first one to experiment
on the tails of fishes from a standpoint of *morphogenesis*. His
work was done largely on *Stenopus, Fundulus*, and *Carassius;* his
findings were published in 1900, 1902, and 1906. He held the
view that *differentiation* is an expression of certain pressure rela-
tions: Typical form is established and growth is completed when
resulting pressures no longer act as a stimulus on the growing
region; the formed part exerts upon the unformed parts an in-
fluence of pressure. Quoting Morgan, " The failure of the maxi-
mum potential of growth over the more distal parts of an oblique
surface is due directly to the new growth below it not having
reached the same level, and owing to this difference there arises a
pull or tension on the part that retards its maximum possible rate."

MATERIAL AND METHODS.

The nearness of Brown University to the collecting grounds of Narragansett Bay makes it possible to experiment upon *Fundulus heteroclitus* and to keep salt water aquaria with daily changes of water brought in by boats from the collecting grounds. The fresh water forms were in an aquarium that had a daily change of fresh water. Both types of aquaria were aereated by means of an electrically driven air pump and green water plants. Worms, wafers and granulated fish food were the types of food used. The temperature of the water was from 19° to 21° C. At Woods Hole, aquaria with running salt water were used.

Cuts were made with scissors and scapels with reference to base of the scales. (The base of the scales as shown in Text fig. *b.* is important, because of the difference of rate of growth from cuts made at each side of this circular base of scales. A strong *Sodium Chloride* solution was used for the removal of any fungus growth. Mild cases thus treated were cured, but the more pronounced cases resulted in death, though, perhaps, slightly retarded by the treatment.

Observations were largely made on living specimens wrapped in wet cloths under the binocular dissecting microscope. Some observations have been made on individuals preserved in formalin and under low power of the microscope. *Camera lucida* has been employed for some detailed observations on regeneration from squares cut in the tails of *Fundulus* and *Carassius*. Photographs of goldfish were made from chloretoned specimens while the tail was spread out in a thin film of water. The photographs of *Fundulus* were made from preserved specimens. The flesh and scales were scraped off and the skeleton of the tail was stained in *alizarin*. They were cleared and photographed in *oil of wintergreen*. The tails were cut from the body by an oblique cut which had the short end of the cut surface of the vertebral column ventral, and the extended end dorsal. This served to orient the tail readily.
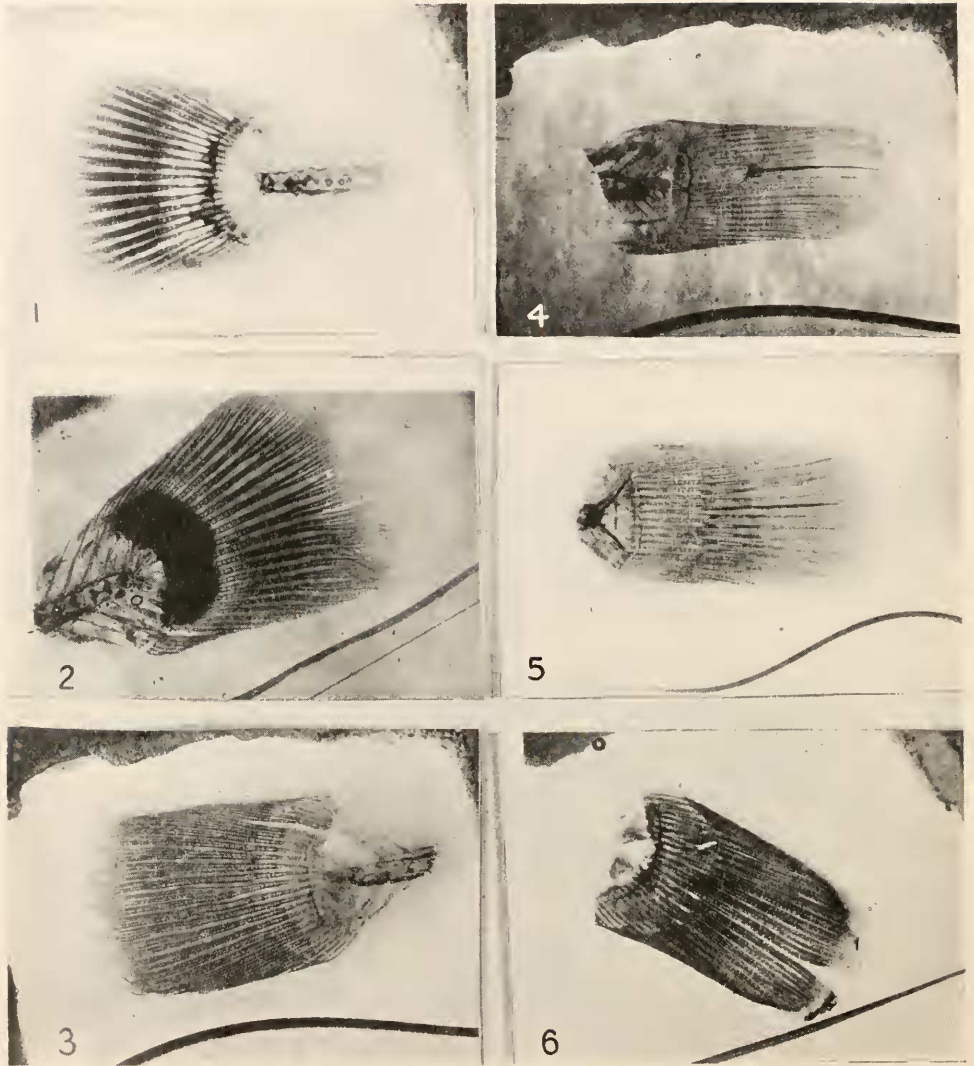
FIGS. 1–6. The shapes or the forms of the tails are correlated with the mode of branching of the fin rays.

FIG. 4. The regenerate from the anterior face of a square hole grew past the posterior face of the hole from which no regeneration had started.

EXPERIMENTAL SECTION.

I.

Description of Tails Used.

The tails of *Fundulus heteroclitus* vary in shape and size (Figs. 1–6). There is a close correlation between the shape of the tail and the mode of branching of the fin rays. The more rounded the tail, the more branched are the fin rays of the tail, especially the rays bordering the dorsal and ventral regions of the tail. The rays articulate with a basal plate at the end of the vertebral column. They are smallest distally and all the rays appear the same size at their distal ends. At the proximal ends the rays are larger than they are at the distal end, and have an articulating knob or enlargement. From the knob the ray becomes smaller until it reaches the base of the scales in the tail; from the *scaly base* it becomes segmented, and more distally there is a doubling of the segments prior to the branching of the rays. This doubling of the segments increases the cross-sectional area of the rays in the middle third of the tail. There is a difference in the number of branching rays in the dorsal and ventral regions of the tail and there is also a difference in position at which the rays of the dorsal and ventral regions branch. The fin rays of the ventral region of the tail branch nearer the scaly base than those of the dorsal region, but more rays of the dorsal region branch than of the ventral region. This difference in mode of branching is already apparent in the early larval stages of the animal, being observed on about the second day after hatching.

In the diagrammatic plate (Fig. 7) it is noticed that the eleventh fin ray from the central one in the dorsal region of the tail doubles its segments and forms secondary branches. The eleventh ray from the central one in the ventral region of the tail does not double and does not form secondary branches. It is readily seen that in this tail more end surface would be exposed in the dorsal region of the tail than in the ventral region, although the cut is supposed to be at the same level in both regions. Neglect of this difference in distribution of fin-ray material gives rise to misleading suggestions as to the controlling factors in growth in tails of fishes,

when distance from base of tail is alone taken as a criterion of level without taking into account these internal structures (see page 252).

The tail of the goldfish differs from that of *Fundulus* in that the largest rays are in the dorsal and ventral lobes and the smallest ones are in the central region (Fig. 8). The rays branch only
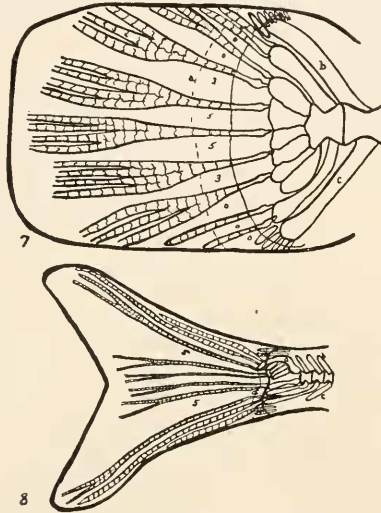

Fig. 7. Diagrammatic and exaggerated sketch of the tail of *Fundulus* which was made from the photograph in Fig. 2. The numbers indicate the omitted rays between any two. (*a*) Scaly base of the tail; (*b*) neural spines; (*c*) hæmal spines.

Fig. 8. Goldfish. (*a*) Urostyle; (*b*) neural spine; (*c*) hæmal spine; (*d*) scaly base.

once instead of twice as in *Fundulus*. Sometimes a goldfish has rays that branch a second time and sometimes in *Fundulus* the rays branch a third time. The shape of the tail is correlated with the mode of branching of the fin rays. The base of the bilobed tail of the goldfish is *heterocercal*, but the tail is *homocercal*. *A. urostyle* projects into the dorsal lobe of the tail. The rays are small near the base of the tail. Prior to branching, the rays enlarge and the segments of the rays divide to form secondary segments. This enlargement of the rays is intimately correlated with the branching of the rays, and occurs in the middle third of the tail. After branching, the rays become small distally. The final

size of all the rays is apparently equal. The rays of the lobes branch after those in the central region of the tail.

The fan-tailed goldfish has the equivalent of two bilobed tails, with the outer rays of the two dorsal lobes being attached along their long axes.

A study of the development of *Fundulus* shows that there is a *primitive natatory fold* and the mesenchymal mass from which the fin rays and the articulating base develop migrates between the extremity of the fold and the end of the notochord and the spinal cord, and from the mesenchymal mass the central rays are the first ones to differentiate. At this time the natatory fold is
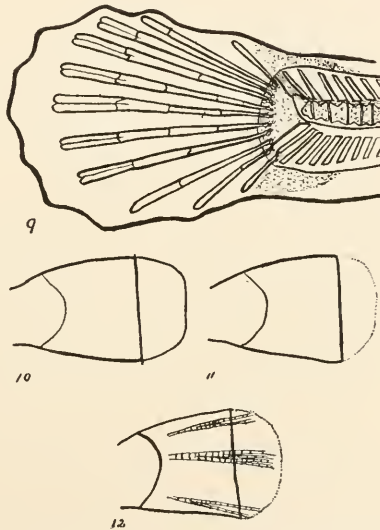

Fig. 9. A camera drawing of the tail at the stage of development when the differences in the distribution of the fin-ray material are first observed. The rays are longer and segmentation is farther from the base in the dorsal region of the tail. The rays in the dorsal region are slightly larger than those of the ventral region. One more ray shows doubling for branching in the dorsal region than in the ventral region.

Figs. 10, 11, 12. This cut was made at the level where there is a transition from large branching rays.

farther from the base of the notochord and the spinal cord at the central region than it is in the dorsal and ventral regions. In individuals that hatched in sixteen days this streaking of the rays begins on the seventh day. The additional rays are added dorsally

and ventrally, but the blood vessels that come to pass between the rays loop in their paths before the rays are stainable vitally with *Nile blue sulphate* or with *alizarin* after fixation. On the eighteenth day (Fig. 9), or two days after hatching, the rays in the dorsal region of the tail become larger and more branched than the rays of the ventral region.

General Features of Regeneration of Tails.

Cut surfaces of various kinds have certain features in common when they regenerate. The proliferation of new tissue, in the forms studied, has proceeded until it is visible at the end of one week in all cases. Cuts at corresponding levels in two different kinds of individuals regenerate at rates that are very close to one another. The rays appear in the new tissue in a period that varies from two to three weeks. The rays can be observed grossly by transmitted light earlier than they can be observed microscopically. This appears to be due to the particular type of condensation in the regenerate before pigmentation begins.

The form of the end of the tail is early observed in all types of regeneration, such as the *diphycercal, homocercal* or fan-tailed fish. The nearer the distal end of the tail that the cut is made, the earlier is the difference in rates of growth observed in the various regions which establish the form of the particular tail. In *Fundulus,* a *primitive diphycercal-tailed* genus, there is an early rounding of the regenerate at its distal extremity. In the *homocercal* tails of the goldfish there is a very early lobing of the tails from cuts near the distal end of the original tail. Pigmentation in the regenerate occurs in most cases after a streaking of the rays is visible. In *Fundulus,* the invasion of the connective tissue regions by pigment is useful in observing the appearance of the rays. In goldfish the gold pigment does not become visible in the regenerate until after the melanophores appear, provided that *melanophores* appear at all. The new tissue can still be distinguished from the old after six months have elapsed.

Regeneration from Cross-Cut Surfaces in the Tails of Fishes.

When *cross-cuts* are made at various levels of the tail (Figs. 10–15), the proliferation of new tissue becomes visible in about

four days. It is apparently equal in amount across the entire sur-
face at the end of the first week. For cut surfaces in the inner
two thirds of the tail, measured from the circular base of scales,
the apparent rates of growth at different levels at which the cuts
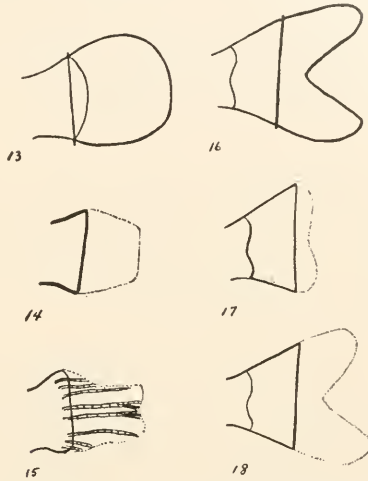
FIGS. 13, 14, 15. This cut was through the region of the tail anterior to
the scaly base. The rays of the center branched once at their distal ends.
The dorsal and the ventral rays did not branch. Five months after the cut
was made, the fish was killed and the sketch made.

FIGS. 16, 17, 18. The different stages of regeneration from a cross cut in
a bilobed tail are pictured.

were made are practically equal. During the first week after
proliferation has become visible, a difference in the rate of growth
between the center of the cut surface and the dorsal and ventral
regions is noticed. In *Fundulus* the tail is rounded up so that the
typical curved form of the tail extremity is approached. In the
lobed-tail goldfish (Figs. 16–18) the typical lobing is very early
approached. In the former case the growth is more rapid at the
center, and in the latter it is more rapid in dorsal and ventral re-
gions of new tissue.

An explanation of the difference in rate in the center of the tail
of *Fundulus* and the dorsal and ventral surfaces on a *cross-cut*
surface may be based on the fact that more cut edge of the osseous
fin rays is exposed in the center of the tail than is exposed dorsally
and ventrally. This is very striking in view of the fact that in

the bilobed tail of the gold-fish the greater quantity of exposed fin-ray material is dorsal and ventral and diminishes toward the center.

The fin rays in the tail of the goldfish are so arranged that the smallest rays are in the center of the tail and the largest rays in the dorsal and ventral lobes of the tail, so that in the *cross-cut* more end surface of fin ray is exposed in the dorsal and ventral regions than in the center. This accounts for the early lobing of the tail.

REGENERATION FROM ANGULAR AND PARTIAL CUT SURFACES.

The regeneration from a *partial cut* or *angular cut* in general is not very different from that of other types of cuts, except that a more limited area of the tail is involved.
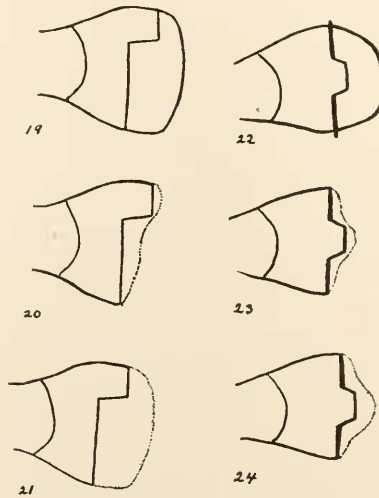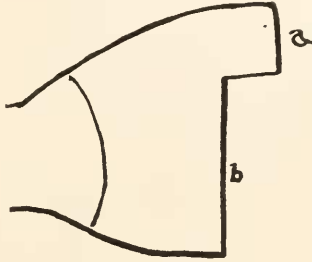

TEXT FIG. *e*. Regeneration from partial surfaces in *Fundulus*.

FIGS. 19, 20, 21. Regeneration from a small dorsal and distal partial surface is slower than that from the central proximal cut.

FIGS. 22, 23, 24. The retardation in regeneration from a central distal and partial surface is not as great as that from a dorsal distal and partial surface.

In *Fundulus* cut so that the distal part of the partial surface (Figs. 19–21) (*a*) (Text Diagram *a*) is dorsal to and smaller than the proximal ventral surface (*b*), growth is more rapid from the latter surface (*b*) than from the former surface (*a*), and the

most rapid growth on the proximal ventral cut is in the region of the central rays of the tail. The proximal surface regenerated faster than the distal surface, and when the rounded distal end of the tail was formed, they proceeded together to complete the parts of the tail then lacking.
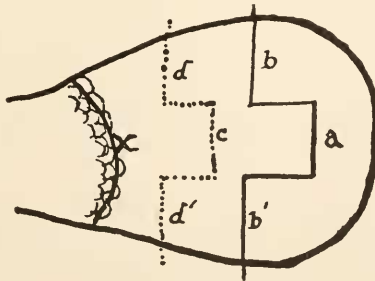

TEXT DIAGRAM *a*.

Quoting Morgan (1906), from page 482:

" The rate of regeneration from the outer *partial cut* surface is greater the broader, *i.e.*, the higher dorso-ventrally, the cut surface. This result shows that the retardation is directly connected with the height of the cut surface, and only secondarily with its distance from the base of the tail."

In other words, Morgan finds it necessary to conclude that tall *partial cuts* are less influenced by pressures and tensions than small ones. But it seems more likely that the regeneration is faster from the tall *partial cuts* because more rays are cut and more surface is exposed than in a smaller cut.

When there are three approximately equal partial cuts (Text Diagram *b*) with the portion including the central rays distal, and

TEXT DIAGRAM *b*.

the ventral and dorsal portions proximal, two types of regeneration are obtained.

When the *partial cuts* are made so that the distal surface (*a*) is through the tertiary branches of the central rays and the proximal surfaces (*b* and *b'*) are through secondary branches of rays that are adjacent to the central ones, the rate of growth is more rapid from the proximal surfaces (*b* and *b'*). In such a case, the rate is slowest from the rays that border the dorsal and ventral surfaces. When the cut is made so that the partial distal surface (*c*) passes through the central rays before they branch and the proximal surfaces (*d* and *d'*) are nearer the scaly base of the tail (*x*), then regeneration is faster from the distal surface (*c*) than from the proximal surfaces (*d* and *d'*). In neither case is the distal central surface retarded in its rate of growth until the proximal surfaces have regenerated enough material to form a rounded distal end.

The results from the three equal *partial cuts* seem to be due to the length of the distal partial surface; for if the distal cut surface is such that the end surface of the rays is exposed at their largest point or some point larger than the point of the dorsal or ventral rays, the distal surface will regenerate faster. But if the distal edge is at a point where the rays are smaller and less surface is exposed than in the proximal regions, then the rates will be reversed.

When a *dorsal distal partial cut surface* is compared with a *ventral distal partial cut surface* at apparently the same level in the tail (Figs. 25–27), the rate of growth, if the level of comparison is near the scaly base of the tail, is faster in the *ventral distal partial cut surface* than it is in the *dorsal distal partial cut surface*. But if the level of comparison is in the middle third of the tail, the rate of growth is faster in the *dorsal distal partial cut surface*.

The explanation for this reversal in the rate of growth between the dorsal and ventral regions lies in the fact that the distribution of the larger amount of fin-ray substance is reversed in the two regions. The rays branch nearer the scaly base in the ventral region than in the dorsal region of the tail, but more fin rays branch in the dorsal region. Therefore, near the base of the tail the rate of regeneration is faster in the ventral region because

the cut was made through the point of doubling of segments prior to branching in the ventral region, whereas this doubling of segments prior to branching had not started in the dorsal region of
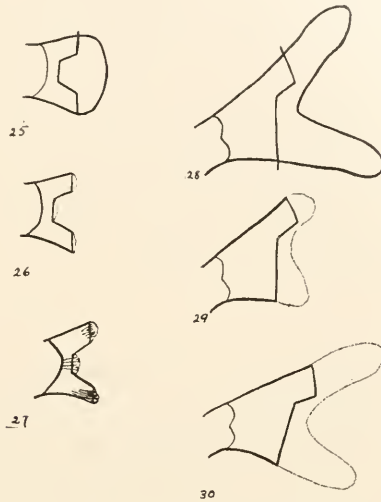
TEXT FIG. *f*. Regeneration from partial surfaces in *Fundulus* and in a bilobed goldfish.

FIGS. 25, 26, 27. The regeneration from two free partial distal surfaces in the tail of *Fundulus* shows regeneration faster dorsally than ventrally. The cuts were made as far as it appeared in the same region and level in the tail. As far as the rays are concerned, the cuts are made at two different levels, with more secondary branches cut dorsally than are cut ventrally.

FIGS. 28, 29, 30. Regeneration from a small distal and dorsal partial surface is not retarded by the more proximal surface. Regeneration from the ventral region of proximal surface is faster than that from the central region of the same surface.

the tail. But in the middle third of the tail the level of the cut passes through the point of doubling of the segments of the primary branches or through the secondary branches, whereas in the ventral region the plane passes through the point of doubling of the segments of the secondary branches or through the tertiary branches.

From a *distal dorsal partial cut* as (*a*) in Text Fig. *a,* in the tail of a goldfish (Figs. 28–30), the rate of regeneration from the distal surface is more rapid than the rate from the proximal surface. There is no " *holding back* " on the free distal partial sur-

face. From a free distal central surface as (*a*) in Text Fig. *b*, the rate of regeneration is less rapid than from the proximal partial surfaces (*b* and *b'*). In this case, the "*holding back*" or retardation of growth is on the central region of the tail.

In the bilobed tail of the goldfish, *partial cuts* regenerate at rates opposite to those of *Fundulus*, the rates being faster dorsally and ventrally where the rays are larger when the *partial cuts* in these regions are either proximal or distal to the central region. Just as in *Fundulus*, the initial rate is about equal, and if the cut passes through the branching region of the central rays, and the other cuts through the distal ends of the dorsal and ventral rays, the rate is faster from the central rays.
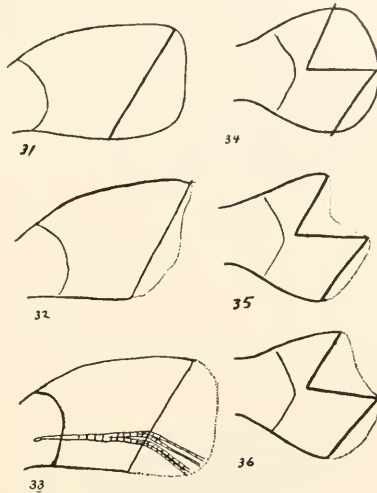
Regeneration from Oblique Cut Surfaces.

From *oblique cuts* in the tail of *Fundulus*, the rate of growth is faster in the proximal region of the cut and slower in the distal region (Figs. 31–33). The new rays from the *oblique cut* stand at an angle with the cut surface that approaches a right angle, and it is from five to six months before the necessary adjustment for straightening the rays occurs.

The visible change in the adjusting regenerate of the tail of the fish is a closer compacting of the regenerated ray with the cut edge of the old ray and an apparent increase of rigidity of new rays as they become more like the old rays. This compacting and increase of rigidity of new rays may explain the pulling of the new part into the axis of the removed part. The compacting of new rays on the ends of the old rays and the gradual becoming rigid of the new ones, exerts a leverage influence on the regenerate. The power and fulcrum are nearly at the same point, hence a rather slow pulling into line of the new part. This adjustment takes from five to six months from a marked oblique cut in *Fundulus*.

The exposure of the end substance by an *oblique cut* would explain the fact that the new tail is at an angle with the old, if it is true that the ends of the rays initiate growth in the tails of fishes. The end of the ray is cut at an angle that exposes an oblique surface from which the new tissue would grow at the rate that it would from a transverse cut across the surface at the same level.

When Morgan noticed, as a result of *partial cuts* in the tail of the same fish, that there was a more rapid rate of growth of the part nearer the base of the tail, he stated that the factors

TEXT FIG. *g.* Regeneration from oblique cuts in the tail of *Fundulus.*

FIGS. 31, 32, 33. The rate of growth from an obliquely cut surface is faster proximally than it is distally. More fin-ray end substance is exposed where the rate of growth is faster.

FIGS. 34, 35, 36. When two oblique cuts are so made that the distal end of dorsal surface is the same distance from the base of the tail as the proximal end of the ventral surface, the rate of growth is faster at the latter. The two points of comparison are not at the same level in terms of the amount of exposed fin-ray material. More secondary rays are cut across in the proximal region of the ventral surface. In the proximal region of the dorsal oblique surface more ray stubs are exposed than in either of the two surfaces; from there the rate of growth is faster.

there were different from those controlling the rate from an oblique cut surface. By means of a series of elaborate *oblique cuts* and *partial cuts,* he set out in 1902 to prove this point. In 1906 he had satisfactorily proved it, although he left an opening for modification of the theory as long as the general principles were retained.

In *Fundulus,* the slow proximal rate of growth in the dorsal or ventral *oblique cuts* can be explained by the fact that the fin rays severed by the proximal cut are first or second order rays, while the distal ones are third order rays. Also that in some

cases, the proximal end passes through the point of branching of some rays. In some cases even here, the regeneration is faster in the center of the *oblique cut*. The type of regenerate is dependent upon the points at which the cut begins and ends. Tails of *Fundulus* vary in the time of branching of the rays and and various shapes of tails are produced by regeneration which depends on the mode of branching of the fin rays.

A marked *oblique cut* of a fin ray which exposes more end cut surface than a transversely cut fin ray at the same level will regenerate more new ray stuff than the transversely cut ray. If the normal pattern of branching is for two orders of fin rays, primary and secondary ones, it is possible for the new ray material from the obliquely cut ray to differentiate into three orders of rays. Figures 37 and 38 show the regeneration from obliquely cut fin rays in the ventral lobe of a goldfish compared
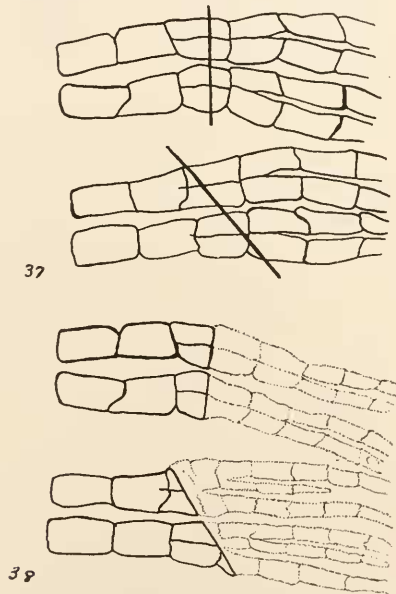

TEXT FIG. *h.* Comparison of regeneration of an oblique and a crosscut in rays.

FIGS. 37, 38. The rays in the dorsal lobe of the tail were cut transversely and those of the ventral lobe were cut obliquely. The end surface exposed by oblique cut was the greater. From the oblique surface, additional secondary rays or branches were formed in the regenerate and from the transverse surface only the normal number of rays regenerated.

with the regeneration from transversely cut rays in the dorsal lobe at the same level in the same tail.

The regenerated ray from the oblique cut may be larger than the ray which gives rise to it. This shows that the cut surface regenerates along its entire surface. More fin-ray end sub-
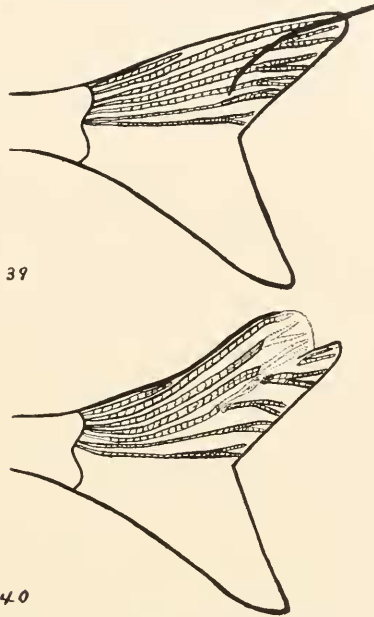
TEXT FIG. *i*. Regeneration from oblique cut in the dorsal lobe of the tail of the goldfish.

FIGS. 39, 40. Regeneration from an oblique cut in the dorsal lobe of the tail of a goldfish. An extra lobe is formed in the tail by rays that grow up and around their dissevered posterior mates.

stance was exposed. The view that the fin ray exerts the initiating influence is in harmony with the theory of Morgan in that he admits that the fin ray must be cut so that an end is exposed in order for regeneration to take place.

The shape of the tail can be altered by an *oblique cut* into the lobe of the tail when no part is removed. There is healing and also regeneration of rays from both sides of the cut. The rays from the anterior face of the cut grow upwards and around the severed posterior rays to form a new lobe in the tail (Figs. 39 and 40).

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The form or shape of the tail does not appear to depend upon any pressure or tension regulating system. The fin rays determine the shape of the tail by their mode of branching and the direction of their growth.

When *double oblique cuts* are made so that the proximal level of the ventral cut is on a perpendicular axis with the distal level of the dorsal cut surface, the rate of growth is faster in the proximal region of the ventral cut surface than it is in the distal region of the dorsal cut surface (Text Diagram *c*) (Figs. 34–36).
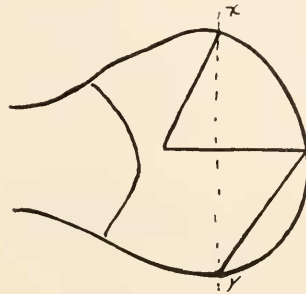
TEXT DIAGRAM *c*.

The most convincing evidence by Morgan that the rate of growth from oblique surfaces was not due to the nearness of one part of the base of the tail, was a cut in which two oblique surfaces were made in the same tail; neither cut directly connected with the other, and the distal end of one cut at the same level as the proximal end of the other. In such a case, the regeneration from the surfaces at the same level were not so close together. His conclusion is that the proximal end exerts a retarding tension on the more distal level of an oblique cut.

In *Fundulus,* the rays are larger in the center of the tail and by normal branching, the central rays reach the minimum size much farther from the base than do the outer fin rays. From double oblique cuts, as before-mentioned, regeneration at the same level of the tail was faster on the proximal surface of one cut than at the distal surface on the other cut. In *Fundulus,* the longitudinal cut in this experiment is adjacent to, or includes the largest rays of the tail, the central rays. The dorsal oblique sur-

face at its distal end is at the end of the final branches of the first (about five) few rays coming from the base of the tail, and at its proximal end intersects the longitudinal cut and the central rays. The ventral oblique surface has its proximal end distal to the final branches of the fin rays that leave the base of the tail. The dorsal cut passes through the smallest branches of the central rays at its distal end and increasingly larger rays towards its proximal end. Hence growth is faster proximally and slower distally. The ventral cut passes through the larger rays proximally and the smaller ones distally. The rate of growth is faster proximally. Further, the rays are correspondingly smaller in most tails at the level of the proximo distal axis (x, y —Text Diagram c) in the dorsal region than in the ventral one. The rays branch nearer the base in the ventral region than in the dorsal, but more rays branch in the dorsal than in the ventral. They tend to reach their minimum size sooner. The middle of the ventral oblique cut is really its fastest point of growth. Moreover, there is a much greater rate of growth at the proximal end of the dorsal cut than at the proximal end of the ventral cut. This is apparently due to the visible difference in size of the rays and the quantity of exposed surface. The rays are different at the two points (x, y) compared.

Assuming the *formative influence* of the rays themselves, and that quantity of cross-cut ray material determines the rate of growth, it will be seen that the same factors controlling the rate of growth from the cross surfaces in the tails of fishes govern the growth from the oblique surface.

Regeneration from an obliquely cut surface in a bilobed tail is faster in the dorsal and ventral lobes when the cut is only slightly oblique. Marked *oblique cuts* regenerate fastest at the proximal end of the cut, and slowest at the center of the cut (Figs. 41, 42, 43). At some points the rate of growth is faster at the center of the cut than at the distal end. Lobing of the tail in the oblique cut is noticed almost as early as in the case of the cross cut in the bilobed-tail fish. The first proliferation of tissue in *oblique cuts* which is observed in about a week seems equal in rate along the entire cut surface. The general tendency, however, is for the rate to be faster at the proximal end, but this is

not pronounced until after the second week. The lobing occurs much earlier when the oblique cut is nearer the original point of lobing of the tail, though when even more than half of the tail is removed, the lobing is noticed before that quantity of new tissue has been restored.
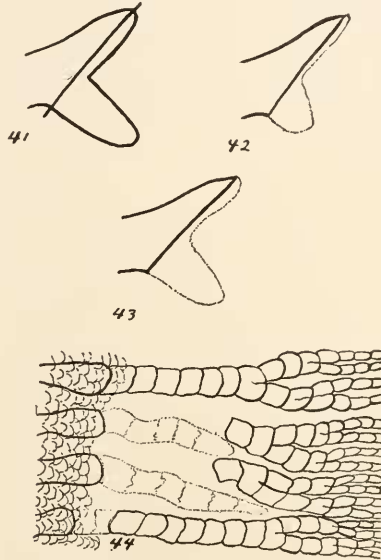
TEXT FIG. *j*. Regeneration from obliquely cut surfaces in the tail of a goldfish and regeneration in a small hole cut in the tail of *Fundulus*.

FIGS. 41, 42, 43. The regeneration from an obliquely cut surface is faster distally than it is in the more proximal central region. The rate of growth is fastest at the most proximal region. The most distal and proximal regions are regions where the rays are larger and where the cut exposes more end substance. More end substance is exposed at the proximal end than at the distal one.

FIG. 44. The new rays from the anterior face of the hole do not connect with their mates at the posterior face of the hole (*Fundulus*).

In the bilobed *Carassius* (goldfish) the rays in the center reach their minimum size first, while the dorsal and ventral rays are much larger, hence much longer. The smaller the branches of the rays are, the slower is the rate of growth in the region of their cut surfaces.

When a *double oblique cut* is made in *Carassius*, so that the distal end of one is at the level of the proximal end of the other in the bilobed tail, the rate of growth is faster at the proximal end.

In some cases, however, the cut can be made so that the distal end of the *dorsal oblique cut* regenerates at practically the same rate as the proximal end of the *ventral oblique cut*.

In the bilobed tail, the longitudinal cut is adjacent to, or includes, the smallest rays in the tail and hence the shortest ones. The distal end of the dorsal *oblique cut* is at the same level as the proximal end of the ventral oblique cut. The distal end of the ventral cut intersects the distal end of the longitudinal cut. The proximal end of the dorsal cut intersects the longitudinal cut near the point of branching of the central rays. The point of the most rapid regeneration is on a line extended directly from the outer ventral ray which is the largest ventral ray, and the most proximal point of the cut. The proximal point on the dorsal cut sometimes shows a more rapid rate of growth than the more distal points on the same surface.

The outer ray, while itself larger than any one ray, does not divide, or if it does, not as early as the rays on the inner surfaces. Cuts through a ray just at the point of division expose more surface as a result than do cuts of a single ray, and that which is not at the point of division is slightly larger than the one that is dividing. Near this is a region where the rate again is faster on the ray that has not reached its minimum in size, for the branches after separating do not expose as much surface as the two when they first separate. Whenever the proximal end of the dorsal oblique cut is distal to the point of branching of the central rays of the bilobed tail, the regeneration is faster distally than it is proximally. Morgan's theory would assume that suddenly a region of great retarding tension was reached that held back the center and the proximal end of the oblique cut. It seems, however, that the rays near the center are smaller, hence do not have to grow so far to attain minimum size.

The mode of growth is just the opposite in the bilobed goldfish type from the type of *Fundulus* and seems to be associated with the fact that the fin rays are just the opposite in size arrangement.

In the elaborate fan-tailed goldfishes the longer parts in the normal tail are supported by the larger rays. The regeneration

is faster where the cut exposed the greater amount of cut end
substance. When two parts of such a tail—one directed pos-
teriorly and one directed ventrally, are cut off, it was observed
that both regenerated equally. Normally, the two parts had ap-
proximately equal lengths, and there is a very close correlation
between the sizes of the rays. They regenerated equally in rate
or with no noticeable difference.

When an *oblique cut* was made of a fan-tail fish so as to re-
move the entire piece that is ventrally directed and the outer
margin of the adjacent posteriorly directed part so as to present
an unbroken cut surface, regeneration took place faster prox-
imally than distally. Examination of the tail showed that the
proximal cut passed through the main body of the branches of
the first order and that the distal cut passed through the distal
ends of the branches of the second order. In the fan-tailed
forms as in the bilobed forms, two orders of branches is the
typical condition. Hence regeneration takes place more rapidly
from the cut ends of rays where more ray surface is exposed.

Regeneration in Square Cuts in the Tail Fins of Fishes.

Small squares cut in the tails of fishes provided a very suitable
means of studying regeneration from four surfaces in the same
tail—two longitudinal and two transverse surfaces.

Regeneration takes place only from the two transverse sur-
faces of the square and only healing takes place along the longi-
tudinal surfaces. Cuts nearer the base are better in that they
can be made much larger and do not break as easily as the more
distal ones. In small cuts 0.2 × 0.2 cm. square, regeneration
takes place by a multiplication of cells around the cut ends of
the fin rays and gradually filling in along the longitudinal sur-
faces by migration from the cross-cut surfaces until a compact
mass completely fills the cut square. From the anterior cut sur-
face of the square and from the ends of the cut rays the new
rays appear during the third week following the cut. No rays
streak out from the posterior or distal face of the square. Some-
times the rays do not unite with their mates, but become dis-
torted or continue to grow and encroach upon the connective
tissue region distal to the posterior face of the square (Fig. 44).

But usually the rays unite with their mates at the posterior surface and form the original missing number of segments (Figs. 45–47). Small fragments of rays broken in cutting migrate to the surface of the cut and are eliminated by rupturing the surface of the closed wound or the regenerated tissue.
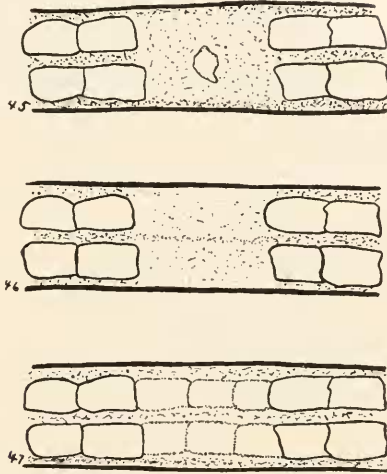
TEXT FIG. *k*. Regeneration from a small hole cut in the tail of goldfish.
FIG. 45. Granulation tissue before hole is completely closed.
FIG. 46. After closure of hole.
FIG. 47. The new rays united with their mates.

From squares of 0.4 × 0.4 cms. a larger breadth and length, regeneration may occur from the posterior and anterior face of the square (Figs. 48–49). The hole never closes and from the anterior face rays continue to grow beyond the posterior face of the cut, in some cases to the original length of the tail. From the posterior face, the maximum length of the regenerated rays so far obtained in *Fundulus* has been eight segments. In several cases of large cuts no growth started from the distal face of the cut and the proximal face produced rays before reaching the distal face. In these cases the proximal growth continued beyond the distal face and did not connect with it. On the distal face there was only healing over. There was no regeneration along the longitudinal surfaces of the square (Fig. 4).

In a goldfish in which a large square had been cut and which
subsequently broke, some important facts were observed.   In
the breaking of the square two posterior faces were left intact
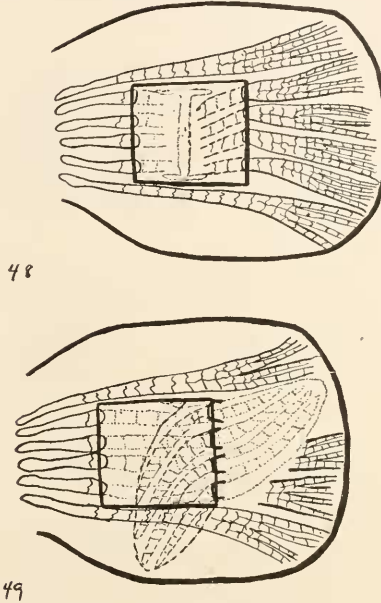
TEXT FIG. *l.* Regeneration in large holes cut in the tails of *Fundulus.*
FIGS. 48, 49. The rays from the anterior and from the posterior face of
the square have regenerated. The rays from one face of the square pass
out to one side of the tail and those from the face pass out to the other.
Eight segments have been formed in the reversed direction.

as only the center broke out (Fig. 50).   Regeneration was
faster from the anterior face and rays of the regenerate curved
around the edges of the posterior faces from which new tissue
was just proliferating.   These rays continued growing to form
the center of the tail and also three rays in the dorsal lobe of the
tail and two in the ventral lobe.   At the end of 45 days, eight
segments had been formed in the reversed growth from the pos-
terior face of the square.   By 73 days after cutting, the rays
from the posterior face of the square had reached the same
number of branches as were in the part distal to the posterior
face of the cut.   A complete reproduction of the skeletal ele-
ments from the posterior face of the square was obtained, as
would have been the case had a simple cross cut been made at
that level.

The form of the lobing of the tail was altered in the before-mentioned goldfish. The normal lobing of the tail was altered to form a lobe within a lobe, as is shown in Fig. 50. The lobe of the tail was altered by the rays from the anterior face of the square that were originally the first rays of the ventral and dorsal lobes. These rays continued in their growth until they reached their limit; their limit was beyond the point of the original center of the tail, even after curving to enter the central portion. The original central rays ceased to grow at their orig-
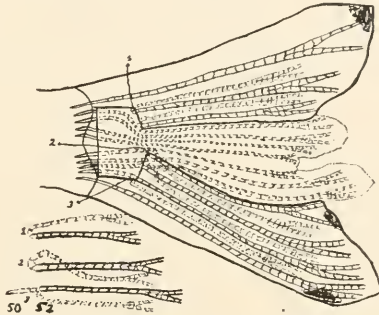

TEXT FIG. *m.* Regeneration in a large hole cut in the tail of a goldfish.

FIG. 50. A drawing of a tail with a large square that had its distal center broken out. (1 and 2) Enlargements of two rays that reversed in the opposite direction. (3) A ray growing from the anterior face of the square unites with a reversed ray from the posterior face of the square at the point of the reversal.

inal limit and a lobing was formed between them and the distorted rays. A second lobing may be said to exist between the distorted and the original lobes. Growth through the center has no retarding influence on rays that normally belong in the lobes.

Reversal of rays in their direction of growth in square holes and formation of all the branches that would have been formed if a cross cut had been made at the level indicate that the power to regenerate and differentiate rests within the fin rays themselves, for they were completely disconnected from the base of the tail. One ray from the anterior face of the hole connected with a ray with reversed growth. The ray from the anterior surface ceased growing and the one from the posterior surface which was nearly complete continued to completion; (the reversed part exits from

between the two rays at their point of fusion in a somewhat lateral direction). *Cessation of growth* in the anterior ray seems related with the fact that its surface was no longer exposed, for it had not yet reached the minimum size, and would have continued to grow if it had not met the ray from the posterior face
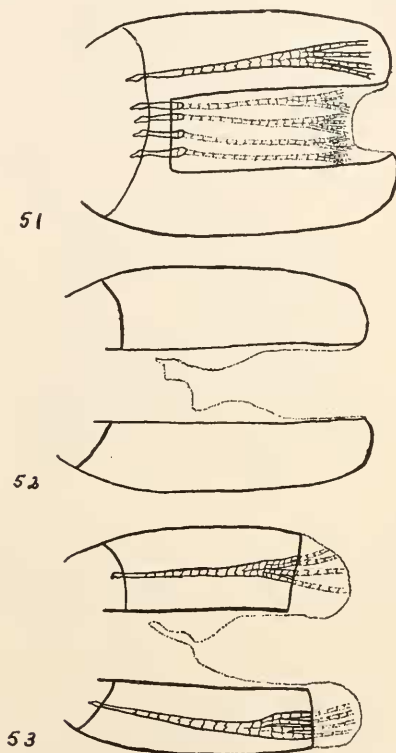
TEXT FIG. *n*. Regeneration after picking out the rays in a given region of the tail in *Fundulus*.

(Comparisons were made after four and one-half weeks.)

FIG. 51. Four central stubs were left in the tail. The new rays branched and the regenerate filled out space made by the cut.

FIG. 52. The entire four central rays were removed and only unformed connective tissue returned. Alizarin staining did not show any rays. The base of the tail was not injured.

FIG. 53. The entire four central rays were removed. Staining showed small rays coming out from the articulating basal plate. There was no form in the regenerate. The base was broken in removing the rays. From cross cuts in the remaining portion of the tail, rays regenerated in three weeks and were visible without staining.

of the hole.   It is not obvious how Morgan's pressure theory would explain these phenomena.

Regeneration after Removal of the Fin Rays.

Regeneration of the tail when some of the fin rays are removed has been obtained (Figs. 51–53).   The rays are picked from the tail in a given region with forceps and a hole is thus made in the tail, as the adjoining tissue is also removed except in the muscle region of the tail.   Healing of the wound may take place. If the rays in a goldfish are removed so that a central small ray is left adjacent to a long ray of the lobe, healing may take place so that the quantity of tissue added is the same as that between any two normal rays.

If a very large area from the tail is removed by picking the rays out from the base, a permanent separation in the lobe persists until new tissue grows out from the base.   If a transverse cut is made in the portion of the tail in which the rays are intact at the same time that the rays are picked out, the rate of growth and establishment of typical form is faster by weeks than the rate of growth and establishment of form in the region from which the rays were removed.   It does appear, however, that it is possible for new rays to grow out from the base when the old ones have been removed.   This has been true in three fish—one *Fundulus* and two goldfish.   Many fish, however, have not regenerated rays.   It is difficult to make sure in such an operation whether a piece of ray has been left or whether the base of the tail has been injured, either of which conditions may account for new rays being produced.   It appears from the stained and cleared tail that the new rays replacing those completely picked out in the tail of *Fundulus* came from the articulating base which was injured in the removal of the old rays.

Discussion.

Broussonet first showed that if the dorsal fin was cut off so that none of the " *osselets* " is left, wound closure takes place by a closing of the cut surface by the *ectoderm*, but no regeneration occurs.   Morgan and others have repeated the experiment with the same results.   The tail-fin will not regenerate if all the fin

rays are cut off by means of a cut anterior to their articulation
with the neural and hæmal spines of the vertebral column.

When a ray is split by means of a longitudinal cut it repairs
itself, but there is no lateral regeneration of the ray substance
(Morgan). The surrounding tissue is simply replaced between
it and the adjacent ray. When new growth from a partial cross
cut reaches a split and repaired ray which has no external adja-
cent mate, then and only then is there a filling-in of new ma-
terial in a lateral direction along the longitudinal cut. Morgan
states that the ray must be cut across if regeneration is to occur;
regeneration from ventral, pectoral and dorsal fins shows that
growth occurs in any direction and any plane from a cross cut.
Additional evidence for regeneration from a cross cut in any di-
rection is presented as a result of a reversal of growth from the
normal to the opposite direction in the tail-fin from cross-cut
faces of a large square.

Ryder (1882) in discussing the development of the tail in fishes
points out that in *Alosa* and in *Pomalobus* the tail is fan-shaped
before the rays are developed, whereas in *Salmo* and *Oncho-
rynchus* the fan-shape is not developed until after the rays have
appeared. In *Gambusia, Siphostoma* and *Hippocampus* there is
no primitive natatory fold from which the tail develops.

Braus (1906) showed that in embryonic *Elasmobranchs,* the
cartilaginous fin rays would develop independently of the muscle
buds of the fin by separation and transplantation of the fin-ray
bud. This was also shown to be the case in some *teleosts.*

Harrison (1925) and Detwiler (1925) have shown in *Ambly-
stoma* that the forelimb develops from a *self-differentiating* and
equipotential system. The *mesenchymal anlage* may be trans-
planted and it will differentiate into a limb without its usual
nerve supply and dependent upon blood for nourishment only.
Detwiler even showed that the development of the forelimb is
not dependent on the shoulder girdle. They suggested that the
growth of the limb may be controlled by the distance of a part
from the center of the bud; after arriving at a certain distance
from the center of the anlage, growth would cease. Other mes-
enchyme could, however, in a restricted sense, due to varying
potencies, simulate the extirpated anlage and produce an ap-

parently normal limb. The explanation of these experiments is based on the *mesenchyme* as a formative factor in growth and seems to be in the same category as the explanation offered for the types of regeneration in the tails of fishes.

To Morgan, the early laying down of the distal form of the tail is due directly to differences in tensions and pressure relations between parts. In *Fundulus* there is a "*holding-back*" tension dorsally and ventrally that produces the rounded tail. In bilobed Carassius the "*holding-back*" tension is greater in the center, and the dorso-ventral surfaces grow more rapidly to form the bilobed tail. From partial cross surfaces at different levels in the same tails, Morgan observed that the rate nearer the base of the tail was slightly faster than at a more distal cut surface. Quoting Morgan: "From this evidence there does not seem to be any doubt that when two *cross-cut* surfaces are present on the same tail, the new part generally grows somewhat faster from the inner of the two surfaces. Comparing this result with the growth when the whole tail is cut off squarely, the conclusion seems highly probable that the differences in the rate of growth over the outer and inner cut surface of the same tail is due to the region of the cut, and not to a regulative influence of one region on the other. This factor may also be present in the regeneration from an oblique surface, but in addition there is also present a regulative influence that holds in check the regeneration from the more distal parts of the new tail."

Barfurth (Morgan, 1902) performed some experiments on tadpoles by means of oblique cuts, and on the regenerate from such cuts, to find the forces that bring the regenerate into the position of the removed part. He observed that from oblique cuts the regenerate makes an angle with the oblique surface that approaches a right angle. Barfurth believes that swimming caused the regenerate eventually to come in line with the old part. By tying down regenerating tadpoles, to prevent swimming, some made the adjustment, but some did not. The evidence that he presented is not conclusive.

Inasmuch as the fin rays are of a mesenchymatous origin, and because of the independence of the muscle buds of cartilaginous rays in *Elasmobranchs* and some teleosts, it seems that the possi-

bility of the fin rays themselves playing a formative rôle in growth rate and morphogenesis in the tail-fins is more than probable. An explanation on such a basis was arrived at as a natural result of an attempt to apply several formative systems and theories that have been suggested; namely, the nervous system, circulatory system, distance from the base of the tail, *axial gradient of metabolism*, and the *tension regulating* mechanism of Morgan.

When the mesenchymatous tail-fin anlage differentiates into rays and connective tissue, the contained formative influences could either be distributed into the formed parts; *e.g.*, to the rays as a result of differentiation, or remain in the tail bud in the form of undifferentiated tissue. But, since regeneration of the tails is due to growth by mitoses at the level of the cut instead of migration, it seems that the result of original embryonic differentiation must be the segregation of the formative influence to the fin rays, or to an interaction of products of differentiation at the level of the cut. Of the two alternatives, the former seems to be the more plausible. The reversal of direction of growth and branching to duplicate the distal parts of the rays from square holes seems to support such a view, for here the rays are disconnected from the base.

The evidence presented in this paper concerning the rate of growth from various types of cuts is essentially in agreement with the results of Morgan. Morgan showed that the fin rays must present an exposed transverse surface in order that regeneration may take place. He thought that the rate of growth in the different regions of the tail is due to differences in tensions and pressures in different parts of the tail, and that proximal ends of oblique cuts exert a " holding-back " tension on the more distal portion of the surface. The form of the tail in various fish is due to differences in pressures and tensions. Evidence has here been presented to show that the fin rays play a formative rôle in the regeneration of the tails of fishes. The mode of branching of the fin rays and the form of the tail are intimately correlated, and regeneration has been shown to take place faster from surfaces where more fin-ray material has been cut across. Braus has shown that the fin-ray anlage will differentiate independently of the muscle bud of the tail, and other mesenchymal

structures have been shown to differentiate independently of the muscular and nervous systems. It is therefore concluded that in the development of the tail of the fish, the rate of growth of the fin comes to be regulated by the size of the rays, and in regeneration the rate of growth and consequently the form is controlled locally by the cross-sectional area of the fin rays exposed.

Summary.

1. Regeneration is faster from the level of the cut that exposes more fin-ray cross surface. The difference in arrangement of the fin rays in the tails of *Fundulus* and goldfish accounts for the rate of growth being faster or slower at opposite regions of the two types of tails.

2. Regeneration from square holes cut in the tails of fishes shows that a reversal of growth of the rays from the posterior face of the hole is possible. Healing in the longitudinal faces and repairing of the injured rays take place. Regeneration takes place only from the cross-cut ends of fin rays. The reversed rays are of the same branching pattern as the distal portion from which they are produced; that is, the same pattern that a cross cut would regenerate posteriorly from that level.

3. When rays that normally would grow into the lobe of the tail are displaced into the central portion of the tail of a goldfish, they are not " held back " by any tension in the center of the tail. The rays continue to grow and form abnormal lobes in the central portion of the tail.

4. Correlation between size and branching is brought out by two types of cuts. (*a*) A cut surface anterior to the scaly base of the tail in *Fundulus* regenerates " abnormal " rays. The shape of the tail can thus be changed from rounded to straight, and persists as such for more than seven months. (*b*) An oblique cut at the point of doubling of rays in the goldfish exposes more cross surface than a straight cross cut. The regenerate from such a surface has produced a third set of branches instead of two sets, as would be typical in the goldfish used.

5. There is a minimum size of all rays in a particular kind of tail, and the size is apparently equal for all the rays.

6. The evidence herein presented suggests that the fin rays are self-differentiating structures.

(*a*) The initiation of growth is due to cutting the fin rays crosswise.

(*b*) Rate of growth seems to depend on the amount of exposed surface of the fin rays.

(*c*) Cessation of growth appears to be due to the attainment of the minimum size of the ray at which no further growth takes place.

(*d*) Form of the tail appears not to be associated with pressure or tension relations but with the mode of branching of the fin rays and hence their size and the formative influences that they possess.

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